Feasibility of novel injectable calcium phosphate cements formulated with bioactive nanocomponent

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Introduction: Calcium phosphates cements (CPCs) have been widely studied in preclinical and clinical scenarios for biocompatible bone replacements or osteogenic substrates for mineralized tissue formation. However, due to relatively low degradability, mechanical strength, and osteogenic properties, the compositional modifications of CPCs have substantially been carried out. Here, we develop novel CPC composition formulated with small incorporations of bioactive nanoparticles. The feasibility of the newly-formulated CPCs for use as promising bone injectables was examined in terms of physico-chemical and mechanical properties as well as in vitro cellular behaviors.

Methods: CPC powder was based on α-TCP. The bioactive nanoparticles were synthesized from the binary glass composition (15CaO-85SiO_2), by means of a sono-reacted sol-gel method. The nanoparticles were added to α-TCP at 0, 2, 5 and 10 wt% to prepare final cement pastes. The setting time was recorded according to the ASTM-C266-08 standard using a Gillmore needle. After setting, the cements were soaked in SBF to observe the phase transformation. The phase and chemical structure of cements were examined by XRD and FT-IR, respectively. The morphology of cements was qualitatively analyzed by SEM. The porosity and surface area of the cements were investigated by porosimeter and BET method, respectively. The compressive mechanical strength was measured by using Instron machine. The protein adsorption study was carried out using cytochrome c as the model protein. Cement samples were soaked in the protein solution, and the adsorbed amount with time was monitored. The in vitro cell adhesion test to the cements was also carried out. Rat MSCs were seeded at 100,000 per sample, and then incubated for 4 h. The dsDNA quantity was measured. The cell adhesion and spreading morphology on the cements were examined by confocal microscopy. The tissue compatibility of the cements was examined in rat subcutaneous tissue.

Results: The setting reaction was significantly enhanced with the incorporation of bioactive nanoparticles. The XRD and FT-IR results showed the formation of bone-like apatite phase in the nanocomposite cement, which was comparable to the phase change in pure CPC. The SEM images showed clear nano-micro-morphological difference in apatite crystal formation between nanocomposite and pure cements. The surface area of the nanocomposite cement was higher than that of pure composition. The compressive mechanical strength was significantly improved by the incorporation of bioactive nanoparticles (Table 1), particularly after immersion in SBF. The adsorption of cytochrome c was significantly higher on the nanocomposite cement than on pure cement, which is believed to result from the enhanced surface area of the nanocomposites. The MSCs cultured on the nanocomposite cements showed significantly enhanced adhesion behaviors, including cell adhesion level and spreading morphology, when compared to those on pure composition. In vivo findings in rat subcutaneous tissue demonstrated excellent tissue responses with no significant inflammatory sings related with the implanted nanocomposite cements.

Conclusions: The novel cements formulated with small additions of bioactive nanoparticles showed excellent physico-chemical and mechanical properties, such as faster setting reaction, higher surface area, and improved compressive strength. Furthermore, the high protein adsorption capacity and the stimulated cellular adhesion, in together with in vivo tissue compatibility, demonstrated the promising use of the novel cement compositions for injectable bone regeneratives.

Table1. Compressive strength of cements before and after immersion in SBF

Parameter	Compressive Strength (MPa)			
	0 %	2 %	5 %	10 %
before immersion	0.9 ± 0.1	1.2 ± 0.2	1.5 ± 0.1	1.8 ± 0.2
After immersion	11.7 ± 1.2	19.6 ± 0.6 **	22.1 ± 2.6 **	25.6 ± 1.7 **

** P<0.01 by ANOVA with Tukey's test

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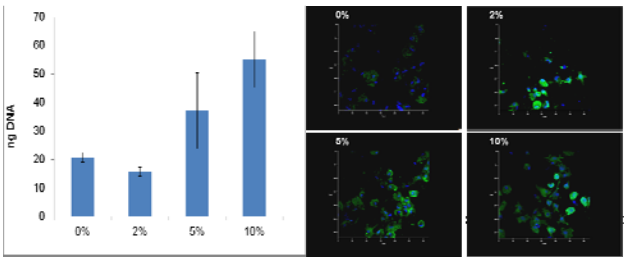

Fig.1. Cell adhesion level and confocal morphology compared between pure and nanocomposite cements.